(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

| (43) International Publication Date | (10) International Publication Number |
| 8 November 2001 (08.11.2001) | **PCT** | **WO 01/84139 A1** |

(51) International Patent Classification[7]: G01N 33/48, 33/50, C12Q 1/68, C12M 3/00

(21) International Application Number: PCT/US01/14223

(22) International Filing Date: 2 May 2001 (02.05.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/208,073 · 4 May 2000 (04.05.2000) US
09/811,762 19 March 2001 (19.03.2001) US

(71) Applicant: THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY [US/US]; 900 Welch Road, Suite 350, Palo Alto, CA 94304-1850 (US).

(72) Inventors: TUSHER, Virginia, Goss; 221 Santa Rita Avenue, Palo Alto, CA 94301 (US). TIBSHIRANI, Robert;

580 St. Claire, Palo Alto, CA 94306 (US). CHU, Gilbert; 3416 Cork Oak Way, Palo Alto, CA 94303 (US).

(74) Agent: HSUE, James, S.; Skejerven Morrill MacPherson, LLP, Three Embarcadero Center, 28th Floor, San Francisco, CA 94111 (US).

(81) Designated States (national): CA, JP.

(84) Designated States (regional): European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR).

Published:
— with international search report
— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: SIGNIFICANCE ANALYSIS OF MICROARRAYS

(57) Abstract: Microarrays can measure the expression of thousands of genes and thus identify changes in expression between different biological states. Methods are needed to determine the significance of these changes, while accounting for the enormous number of genes. We describe a new method, Significance Analysis of Microarrays (SAM), that assigns a score to each gene based on the change in gene expression relative to the standard deviation of repeated measurements. For genes with scores greater than an adjustable threshold, SAM uses permutations of the repeated measurements to estimate the percentage of such genes identified by chance, the false discovery rate (FDR). When the transcriptional response of human cells to ionizing radiation was measured by microarrays, SAM identified 34 genes that changed at least 1.5-fold with an estimated FDR of 12%, compared to FDRs of 60% and 84% using conventional methods of analysis. Of the 34 genes, 19 were involved in cell cycle regulation, and 3 in apoptosis. Surprisingly, 4 nucleotide excision repair genes were induced, suggesting that this repair pathway for UV-damaged DNA might play a heretofore unrecognized role in repairing DNA damaged by ionizing radiation.

SIGNIFICANCE ANALYSIS OF MICROARRAYS

BACKGROUND OF THE INVENTION

5   This invention relates in general to statistical analysis of gene related data and, in particular, to analysis of microarray data for identifying genes that exhibit statistically significant behavior.

Different biological systems are characterized by differences in the copy number of genes or in levels of transcription of particular genes. By measuring such biological

10   phenomena, insight into and possible treatment of human diseases may be found.

Microarrays of various types have been employed for measuring the expression levels of large numbers of genes.   One type of microarray is the oligonucleotide microarray, one example of which is the Gene Chip® microarray manufactured by Affymetrix corporation of California. International Patent Application PCT/US96/14389,

15   which is incorporated herein in its entirety, describes a method for measuring gene expression levels using oligonucleotide microarrays. In the method described, a nucleic acid sample is hybridized to a high density array of oligonucleotide probes immobilized to a surface, where the high density array contains oligonucleotide-type probes complementary to sequences of the target nucleic acids in the nucleic acid sample. For

20   example, RNA transcripts of one or more target genes may be hybridized to an array of oligonucleotide probes immobilized on a surface such as that of a semiconductor chip. Some of the probes on the surface have sequences that are perfectly complementary to particular target sequences and are referred to herein as perfect match (PM) probes. Also present on the chip are probes whose sequence is deliberately selected not to be perfectly

25   complementary to a target sequence. Such probes are referred to as mismatched (MM) control probes, where for each PM probe, there is a MM control probe for the same particular target sequence. This mismatch may comprise one or more bases. Thus, the biological sample such as a mRNA sample can be analyzed for gene expression for hybridization to above-described microarray on a chip. The presence of RNA sequences

30   that bind to the oligonucleotide probes on the chips are then detected by methods such as tagging with a fluorescence material and then detecting the fluorescence.   Since sequences that are different from the target sequences may also bind to the PM probes that correspond to such target sequences, the fluorescence signals from such sequences

would appear as noise. Signal-to-noise ratio is improved by calculating the difference from signals from the sequences that bind to the PM probes and the signals from sequences that bind to the MM probes.

Another type of microarray that has been used for analyzing gene expression utilizes cDNA probes. Although massive amounts of data are generated using oligonucleotide or cDNA probes, quantitative methods are needed to determine whether differences in gene expression are experimentally significant. Previous work on microarrays has utilized cluster analysis, to find coherent in expression patterns among genes or in cells. See, for example, the following three articles:

1.   Alizadeh, A., Eisen, M., Davis, R., Ma, C., Lossos, I., Rosenwal, A., Boldrick, J., Sabet, H., Tran, T., Yu, X., Marti, G., Moore, T., J, H., Lu, L., Lewis, D., Tibshirani, R., Sherlock, G., Chan, W., Greiner, T., Weisenburger, D., Armitage, K., Levy, R., Wilson, W., Greve, M., Byrd, J., Botstein, D., Brown, P. & Staudt, L. (2000) *Nature* **403**, 503-511.

2.   Eisen, M., Spellman, P., Brown, P. & Botstein, D. (1998) *Proc. Natl. Acad. Sci. USA* **95**, 14863-14868.

3.   Weinstein, J., Myers, T., O'Connor, P., Friend, S., Fornace, A., Kohn, K., Fojo, T., Bates, S., Rubinstein, L., Anderson, N., Buolamwini, J., van Osdol, W., Monks, A., Scudiero, D., Sausville, E., Zaharevitz, D., Bunow, B., Viswanadhan, V., Johnson, G., Wittes, R. & Paull, K. (1997) *Science* **275**, 343-349.

Cluster analysis works best for a large number of samples. Moreover, cluster analysis provides little information about statistical significance. To answer biologically important questions, a method is needed which can analyze a relatively small number of samples and provide a measure of statistical certainty. Methods based on conventional t-tests provide the probability (p) that a difference in gene expression occurred by chance. See for example, the following articles:

4.   Roberts, C., Nelson, B., Marton, M., Stoughton, R., Meyer, M., Bennett, H., He, Y., Dai, H., Walker, W., Hughes, T., Tyers, M., Boone, C. & Friend, S. (2000) *Science* **287**, 873-880.

5.   Galitski, T., Saldanha, A., Styles, C., Lander, E. & Fink, G. (1999) *Science* **285**, 251-254.

In conventional t tests, p = 0.01 may be significant in the context of experiments designed to evaluate small numbers of genes. However, a microarray experiment for 10,000 genes would identify 100 genes by chance.

One approach for ascertaining the statistical significance of microarray data is known as the "fold change" method. In this approach, if one were interested in measuring the effects of radiation on gene expression, a number of biological samples are subjected to radiation, and their gene expression is then measured. Other biological

5 samples are measured without being subjected to radiation. The "fold change" method identifies genes as having been changed significantly by the radiation if the ratio of the average gene expression measured after being subjected to the radiation to the gene expression measured without being subjected to radiation is greater than a certain threshold or less then another threshold. As further explained below, the "fold change"

10 method, in some instances, yields unacceptably high false discovery rates.

In one attempt to improve on the "fold change" method, genes are identified to be significantly changed if a certain fold change is observed consistently between paired samples. While this yields a moderate improvement over the "fold change" method, this improved "pair wise fold change" method still yields a rather high false discovery rate.

15 As also noted above, conventional techniques analyze differences in gene expression levels, such as PM-MM, so that negative expression values are possible during analysis. Conventional methods of calculation and graphical representation employ log-log plots which do not permit negative values. Where linear plots are used instead for representing such possible negative values, it is found, however, that most of

20 the values in the plots tend to congregate in a small area so that it is difficult to resolve them visually. It is, therefore, desirable to provide improved techniques for calculation and representation of data.

It is, therefore, desirable to provide an improved system for analyzing and representing data obtained from microarrays whereby the above-described difficulties are

25 alleviated.

SUMMARY OF THE INVENTION

A new method, referred to herein as Significance Analysis of Microarrays (SAM), identifies genes with statistically significant differences in expression or other biological

30 characteristics (such as gene copy number or levels of protein encoded by the genes), referred to below as values associated with the genes, by assimilating a set of gene-specific microarray data. For example, SAM may assign each gene a score representing such associated values, based on differences in gene expression or other biological characteristics in the data relative to the standard deviation of repeated measurements for

that gene. Genes with scores greater than an adjustable threshold are deemed potentially significant. In some situations, gene expression may vary over a wide range of values, so that, in order to take full advantage of statistical analysis, it is preferable to choose statistical parameters for characterizing genes so that statistical significance can be assessed despite such variation of values. Preferably the parameters are chosen so that they are substantially independent of the ranges of values that characterize the genes. Thus, where a plurality of genes are associated with a plurality of sets of values obtained from data sources, a statistical parameter is provided that contains information concerning differences in the associated values of the genes among the sets. In one implementation, the parameters of the genes are adjusted so that the parameters are substantially independent of the average associated values of the genes over the sets. An observed value and an expected value of the adjusted parameter are calculated and compared to identify genes whose associated values differ by an amount of statistical significance among the sets. The sets of associated values of genes may be obtained from measurements using microarrays, data derived from such measurements, calculations or predictions using gene models, or other data sources.

As noted above, gene expression or other biological characteristics of genes may vary over a wide range of values. Therefore, for genes whose expression or other characteristics have high values, even a difference that is a small percentage of the high values may overshadow and mask larger relative differences for genes whose expression or other characteristics have lower values. Furthermore, factors inherent in the process of acquisition of the data analyzed may introduce noise that may mask changes or differences in gene expression, or cause genes to be erroneously identified as having changes of statistical significance. This problem can be alleviated by ranking the genes by their values of the parameter, and by deriving expected values of the parameter of different ranks. The expected value for the parameter for each rank is then compared with the value of the parameter of the gene of the same rank to identify genes that exhibit changes of statistical significance.

In one embodiment, the expected value for the parameter for each rank is obtained by permuting the associated values of genes, deriving a value of such parameter for each gene in each permutation, ranking the values of the parameter, and obtaining an average value of the parameter of each rank for the permutations.

Inherent in some statistical methods such as the one described above is that some genes may be erroneously identified as ones with statistically significant differences in

expression or other characteristics. A good indication of the effectiveness of the method is to compute a false discovery rate for the method.

To estimate the percentage of such genes identified by chance (the false discovery rate, FDR), nonsense genes are identified by analyzing permutations of the measurements. The threshold score can be adjusted to identify smaller or larger sets of genes, and FDRs are calculated for each set.

The FDR may be found by permuting the associated values of genes, deriving a value of such parameter for each permutation, ranking the values of the parameter, and comparing the values of the parameter to a threshold to find the FDR. In one embodiment, this is implemented by counting the number of genes with parameter values that exceed a positive threshold or fall below a negative threshold. One possible method for estimating the FDR is to define FDR as the number of such nonsense genes divided by the number of actual genes with parameter values that exceed the positive threshold or fall below the negative threshold.

Where SAM is used in connection with data analysis of diseases, gene expression or other characteristic values may correlate with patient survival time. In such event, pairs of death and risk sets may be defined, each pair having a corresponding patient death time, where the death set includes associated values corresponding to the death time and the risk set includes values corresponding to times occurring after the death time. A parameter is then provided for each of the genes containing information concerning differences in the associated values of the gene among the sets. An observed and an expected value of the parameter for each gene are then derived and compared to identify genes that exhibit behavior of statistical significance.

To avoid the problem inherent in the conventional technique of using sharp thresholds in deriving representative values of genes, smooth weighting functions may be used to reduce distortion. In order to analyze and/or represent expression levels that may be negative or positive in value, odd root values may be analyzed and/or graphically displayed so that the values do not congregate in a small area in the plot, and this facilitates analysis and comparison.

The above-described features may be embodied as a program of instructions executable by computer to perform the above-described different aspects of the invention. Hence, any of the techniques described above may be performed by means of software components loaded into a computer or any other information appliance or digital device. When so enabled, the computer, appliance or device may then perform the above-

described techniques to assist the analysis of sets of values associated with a plurality of genes in the manner described above, or for comparing such associated values. The software component may be loaded from a fixed media or accessed through a communication medium such as the internet or any other type of computer network. The

5   above features embodied in one or more computer programs may be performed by one or more computers running such program(s).

Each of the inventive features described above may be used individually or in combination in different arrangements. All such combinations and variations are within the scope of the invention.

10

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1A is a linear scatter plot of gene expression in a sample hybridized to two microarrays using a conventional technique, where each gene (i) in the microarray is represented by a point with coordinates consisting of gene expression measured in

15   uninduced cell line 1 from hybridization A, $x_{U1A}(i)$, and gene expression from the same cell line from hybridization B, $x_{U1B}(i)$.

Fig. 1B is a cube root scatter plot of gene expression from the data in Figure 1A to illustrate an aspect of the invention.

Fig. 1C is a cube root scatter plot of average gene expression (avg x_A) from the

20   four A hybridizations (induced and uninduced, cell lines 1 and 2) and the four similar B hybridizations (avg x_B) to illustrate an aspect of the invention.

Fig. 1D is a cube root scatter plot of average gene expression from the four hybridizations with uninduced cells (avg x_U) and induced cells 4 hr after exposure to 5 Gy of IR (avg x_I), where some of the genes that responded to IR are indicated by arrows

25   to illustrate an aspect of the invention.

Figs. 2A-2F are scatter plots of relative difference in gene expression d(i) versus gene specific scatter s(i), where the data were partitioned to calculate d(i) as indicated by the bar codes, and where the shaded and unshaded entries were used for the first and second terms in the numerator of d(i) in Equation 1 set forth below.

30   Fig. 2A illustrates the relative difference between irradiated and unirradiated states, where the statistic d(i) was computed from expression measurements partitioned between irradiated and unirradiated cells.

Fig. 2B illustrates the relative difference between cell lines 1 and 2, where the statistic $d(i)$ was computed from expression measurements partitioned between cell lines 1 and 2.

Fig. 2C illustrates the relative difference between hybridizations A and B, where the statistic $d(i)$ was computed from the permutation in which the expression measurements were partitioned between the equivalent hybridizations A and B.

Figs. 2D, 2E, 2F illustrate the relative differences for three permutations of the data that were balanced between cell lines 1 and 2.

Figs. 3A-3C illustrates a process for identification of genes with significant changes in expression.

Fig. 3A is a scatter plot of the observed relative difference $d(i)$ versus the expected relative difference $d_E(i)$, where the solid line at 45 degrees indicates the line for $d(i) = d_E(i)$, where the observed relative difference is identical to the expected relative difference, and where the dotted lines are drawn at a distance $\Delta = 1.2$ from the solid line.

Fig. 3B is scatter plot of $d(i)$ versus scatter $s(i)$.

Fig. 3C is a cube root scatter plot of average gene expression in induced and uninduced cells, where the cutoffs for 2-fold induction and repression are indicated by the dashed lines, and where in all panels, the 46 potentially significant genes for $\Delta = 1.2$ are indicated by the squares.

Figs. 4A-4C illustrate a process for comparison of SAM to conventional methods for analyzing microarrays.

Fig. 4A illustrates falsely significant genes plotted against number of genes called significant, where of the 57 genes most highly ranked by the fold change method, 5 were included among the 46 genes most highly ranked by SAM.

Fig. 4B is a Northern blot validation for genes identified by the fold change method, where values of $r(i)$ are plotted for genes chosen at random from the 57 genes most highly ranked by the fold change method.

Fig. 4C is a Northern blot validation for genes identified by SAM, where results are plotted for genes chosen at random from the 46 genes most highly ranked by SAM. The straight lines in Figs. 4B and 4C indicate the position of exact agreement between Northern blot and microarray results.

Fig. 5A is a graphical plot of a scatter function to illustrate effects of a conventional technique for processing gene expression which eliminates contributions from probes that diverge from a mean value by a predetermined cutoff.

Fig. 5B is a graphical plot of a scatter function to illustrate effects of the use of a
5   Gaussian weighting function for processing gene expression to illustrate an aspect of the invention.

Fig. 6 is a block diagram showing a representative sample logic device in which aspects of the present invention may be embodied.

For simplicity in description, identical components are labelled in the same
10   numerals in this application.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Because of its biological importance, SAM is applied to the transcriptional response of lymphoblastoid cells to ionizing radiation (IR). Although the data were
15   obtained from oligonucleotide microarrays representing 6800 genes, SAM can also be applied to cDNA microarrays in a similar manner.

Materials and Methods Used in the Invention:

Preparation of RNA. Lymphoblastoid cell lines GM14660 and GM08925
20   (Coriell Cell Repositories, Camden, NJ) were seeded at 2.5 x 10^5 cells/ml and exposed to 5 Gy 24 hours later. RNA was isolated, labeled and hybridized to the HuGeneFL GeneChip® microarray according to manufacturer's protocols (Affymetrix, Santa Clara, CA).

Microarray hybridization. Each gene in the microarray was represented by 20
25   oligonucleotide pairs, each pair consisting of an oligonucleotide perfectly matched to the cDNA sequence and a second oligonucleotide containing a single base mismatch. Because gene expression was computed from differences in hybridization to the matched and mismatched probes, expression levels were sometimes reported by the GeneChip® Analysis Suite software as negative numbers. To compare data from different microarray
30   hybridizations, a reference data set was constructed from the average expression for each gene over the 8 data sets. Gene expression for each hybridization was plotted against the reference data set in a cube root scatter plot and scaled by a linear fit to the data points. Data were then cubed to return values to the original scale.

Northern blot hybridization. Total RNA (15 µg) was resolved by agarose gel electrophoresis, transferred to a nylon membrane, and hybridized to specific DNA probes, which were prepared by PCR amplification.

5   **Results of Applying the Invention to a Biological System:**

RNA was harvested from two wild type human lymphoblastoid cell lines, designated 1 and 2, growing in an unirradiated state U, or in an irradiated state I, 4 hr after exposure to a modest dose of 5 Gy of IR. RNA samples were labeled and divided into two identical aliquots for independent hybridizations, A and B. Thus, data was

10   generated from eight hybridizations (U1A, U1B, U2A, U2B; I1A, I1B, I2A, I2B).

To assess reproducibility in the data, identical aliquots of an mRNA sample (U1A and U1B) were analyzed with two microarrays from the same manufacturing lot. A linear scatter plot for gene expression confirmed that the data was generally reproducible (Figure 1A), but failed to resolve the vast majority of genes that are expressed at low

15   levels. To better resolve these genes, we chose to display the data in a cube root scatter plot. This permitted the inclusion of negative levels of expression that are sometimes generated by the GeneChip® software. The cube root scatter plot (Figure 1B) revealed three salient features: the large percentage of genes (24%) assigned negative levels of expression, the large percentage of genes with low levels of expression, and the low

20   signal to noise ratio at low levels of expression.

Figure 1A is a linear scatter plot of gene expression in a sample hybridized to two microarrays using a conventional technique, where each gene (i) in the microarray is represented by a point with coordinates consisting of gene expression measured in uninduced cell line 1 from hybridization A, $x_{U1A}(i)$, and gene expression in the same cell

25   line from hybridization B, $x_{U1B}(i)$. As can be observed from Figure 1A, only a small number of highly expressed genes are resolved visually, with most of the genes compressed into a small region of the plot so that they would be difficult to resolve visually. One method of distributing such data points more uniformly is a logarithmic scatter plot, but the log function cannot accept the negative values for gene expression

30   generated by the microarrays. Figure 1B is a cube root scatter plot of gene expression from the data in Figure 1A to illustrate an aspect of the invention. As will be clear from a comparison of Figures 1A, 1B, the genes with lower expression levels are more visually resolved in cube root plot of Figure 1B compared to Fig. 1A. While cube root plots are

illustrated herein, it will be understood that the fifth root or other odd root plots may be used instead and are within the scope of the invention.

After scaling the data from different microarray hybridizations, a scatter plot was generated for average gene expression in the four A aliquots vs. the average in the four B aliquots, a partitioning of the data that eliminates biological changes in gene expression. The scatter was improved by averaging multiple data sets (compare Figures 1B and 1C). Figure 1C is a cube root scatter plot of average gene expression from the four A hybridizations (avg x_A) and the four B hybridizations (avg x_B).

To assess the biological effect of IR, a scatter plot was generated for average gene expression in the four irradiated states vs. the four unirradiated states (compare Figures 1C and 1D). Figure 1D is a cube root scatter plot of average gene expression from the four hybridizations with uninduced cells (avg x_U) and induced cells 4 hr after exposure to 5 Gy of IR (avg x_I), where some of the genes that responded to IR are indicated by arrows to illustrate an aspect of the invention. A few of the potentially significant changes in gene expression are indicated by arrows in Figure 1D, but the effect was not easily quantified, and it is desirable to provide a better method to identify changes with a level of statistical confidence.

The approach adopted herein was based on analysis of random fluctuations in the data. In general, the signal to noise ratio decreased with decreasing gene expression (Figures 1B-1D). However, even for a given level of expression, it is found that fluctuations were gene specific. To account for gene-specific fluctuations, a statistic is defined based on the ratio of change in gene expression to standard deviation in the data for that gene. The "relative difference" $d(i)$ in gene expression is:

$$d(i) = [\underline{x}_I(i) - \underline{x}_U(i)]/[x(i) + s_0] \qquad (1)$$

where $\underline{x}_I(i)$ and $\underline{x}_U(i)$ are defined as the average levels of expression for gene (i) in states I and U, respectively. The "gene-specific scatter" $s(i)$ is the standard deviation in the data:

$$s(i) = (\{\textstyle\sum_m [x_m(i) - \underline{x}_I(i)]^2 + \sum_n [x_n(i) - \underline{x}_U(i)]^2\}\{1/n_1 + 1/n_2\}/\{n_1 + n_2 - 2\})^{1/2} \qquad (2)$$

where $\sum_m$ and $\sum_n$ are summations of the expression measurements in states I and U, respectively, and n_1 and n_2 are the numbers of measurements in states I and U (4 in this

experiment). A constant $s_0 = 3.3$ was chosen by minimizing the coefficient of variation of the standard deviation of d(i) as a function of s(i), thus permitting d(i) values to be compared among all genes in the microarray. While a relative difference parameter d(i)
as set forth in equation (1) is preferable, it will be understood that other difference
5   functions that depend on the differences between the associated values of the genes among the sets (e.g. set of measurements in state U and set of same in state I) and on scatter values among the sets may be used and are within the scope of the invention.

As noted above, factors inherent in the process of acquisition of microarray data itself may introduce noise that renders it difficult to discover the significance of
10   differences in gene expression or other biological behavior or falsely identify genes to be of statistical significance. To overcome such problem, a number of methods are described above which allow full utilization of the microarray data. One difficulty in making use of the microarray data is due to the fact that the expression levels of the genes have a wide range of values or scattered values. It is, therefore, desirable to adjust the
15   parameter d(i) so that it is essentially independent of the wide variation of the values of the parameter d(i) and/or of the scatter value s(i). After the parameter has been so adjusted, then all of the data can be fully utilized.

In one embodiment, the adjustment is accomplished by dividing the scatter values or average associated values of the genes into subsets each having a similar range of
20   values. For example, the scatter values or average associated values of the genes may be divided into ten subsets in accordance with which percentile such values fall into. In other words, the first of the ten subsets will contain the top tenth percentile of the scatter values or average associated values of the genes, the second subset containing the second to the top tenth percentile of such values and so on. The standard deviation of the
25   parameter d(i) is then calculated within each subset and a coefficient of variation of the standard deviations of the parameter values for the ten subsets is then minimized by varying the value of the constant s_0 appearing in equation 1. After the constant s_0 has been so adjusted, the parameter d(i) is then substantially independent of wide variations in scatter values or average associated values of the genes, so that all of the microarray
30   data can be effectively used.

Scatter plots of d(i) vs. log[s(i)] are shown in Figure 2A-2F which are scatter plots of relative difference in gene expression d(i) versus gene specific scatter s(i), where the data were partitioned to calculate d(i) as indicated by the bar codes, and where the shaded

and unshaded entries were used for the first and second terms in the numerator of d(i) in Equation 1 set forth below. Figure 2A illustrates the relative difference between irradiated and unirradiated states, where the statistic d(i) was computed from expression measurements partitioned between irradiated and unirradiated cells. By contrast, the

5   scatter plot for relative difference between cell lines 1 and 2 shows more marked changes in Figure 2B, which illustrates the relative difference between cell lines 1 and 2. In Figure 2B, the statistic d(i) was computed from expression measurements partitioned between cell lines 1 and 2. Thus, the relative difference between cell lines 1 and 2 appears to exceed that between irradiated and unirradiated states.

10   These relative differences exceeded random fluctuations in the data, as measured by the relative difference between hybridizations A and B in Figure 2C which illustrates the relative difference between hybridizations A and B. In Figure 2C, the statistic d(i) was computed from the permutation in which the expression measurements were partitioned between the equivalent hybridizations A and B.

15   Although the relative difference computed from hybridizations A and B provided a control for random fluctuations, additional controls were desirable to assign statistical significance to the biological effect of IR. Instead of performing more experiments, which are expensive and labor-intensive, a large number of controls are generated by computing relative differences from permutations of the hybridizations for the 4

20   irradiated and 4 unirradiated states. To minimize potentially confounding effects from differences between the two cell lines, the data was analyzed using the 36 permutations that were balanced for cell lines 1 and 2. Permutations were defined as balanced when each group of four experiments contained two experiments from cell line 1 and two experiments from cell line 2. Figures 2D, 2E, 2F illustrate the relative differences for

25   three permutations of the data that were balanced between cell lines 1 and 2.

Relative differences from random permutations of the hybridizations indicate noise inherent in the process of data acquisition. From the examples illustrated above, it is seen that relative differences stemming from the differences between cell lines may mask statistically significant changes in gene expression caused by radiation, so that for

30   this reason, it may be preferable to use only data from balanced permutations, to reduce the effects on the statistics from differences between the cell lines.

Another control that can be exerted is by ranking the values of the relative difference parameter d(i) Although gene expression levels can vary widely, the relative difference d(i) is a measure of statistical significance substantially independent of

expression level. As another control for assigning statistical significance, the largest relative differences from the 36 permutations may indicate noise from statistical fluctuations in the data. One may compute the average value of the largest relative differences from all 36 permutations. Thus, comparing the largest relative difference among all the genes to the largest relative differences from the permutations provides one possible test for identifying genes to be of statistical significance. Therefore, the average of the largest relative differences from the 36 permutations is the expected relative difference for such gene. A comparison of the relative difference of such gene with its expected value can be used as control as to whether statistical significance should be assigned to such gene. The same reasoning applies to the gene of the second highest relative difference and comparison to the second largest relative differences from the permutations, and so on for all the genes involved in the calculation.

In other words, to find significant changes in gene expression, genes were ranked by magnitude of their $d(i)$ values, so that $d(1)$ is the largest relative difference, $d(2)$ is the second largest relative difference, and $d(i)$ is the ith largest relative difference, or the ith rank. For each of the 36 balanced permutations, relative differences $d_p(i)$ are also calculated, and the genes are again ranked such that $d_p(i)$ was the ith largest relative difference for permutation p. The expected $d_E(i)$ was defined as the average over the 36 balanced permutations,

$$d_E(i) = \Sigma_p d_p(i)/36 \qquad\qquad (3)$$

Figures 3A-3C illustrates a process for identification of genes with significant changes in expression. Figure 3A is a scatter plot of the observed relative difference $d(i)$ versus the expected relative difference $d_E(i)$, in which the solid line indicates the line for $d(i) = d_E(i)$, where the observed relative difference is identical to the expected relative difference, and in which the dotted lines are drawn at a distance $\Delta = 1.2$ from the solid line. Figure 3B is a scatter plot of $d(i)$ versus scatter $s(i)$. Figure 3C is a cube root scatter plot of average gene expression in induced and uninduced cells, where the cutoffs for 2-fold induction and repression are indicated by the dashed lines, and where in all panels, the 46 potentially significant genes for $\Delta = 1.2$ are indicated by the squares.

To identify potentially significant changes in expression, a scatter plot of the observed relative difference $d(i)$ vs. the expected relative difference $d_E(i)$ (Figure 3A) is

used. For the vast majority of genes, d(i) ≅ d$_E$(i). However, some genes are represented by points displaced from the d(i) = d$_E$(i) line by a distance greater than a threshold Δ. For example, the threshold Δ = 1.2 illustrated by the broken lines in Figure 3A yielded 46 genes that were "called significant." These 46 genes are shown in the context of the scatter plot for d(i) vs. log[s(i)] (Figure 3B) and in the scatter plot for the cube root of gene expression $\underline{x}_I$(i) vs. $\underline{x}_U$(i) (Figure 3C). Clearly, genes identified by d(i) do not necessarily have the largest changes in gene expression.

As noted above, the relative differences of the various permutations indicate noise inherent in the data acquisition process. Such relative differences may then be used to determine the number of genes falsely identified to be of statistical significance. False discovery rate may be found by comparing such relative differences to thresholds. Fig. 3A may be used for such purposes as well, where the "observed" relative difference d(i) in the figure is one obtained from permutations as described below.

In one embodiment, to determine the number of falsely significant genes generated by SAM, horizontal cutoffs were defined as the smallest d(i) among the genes called significantly induced and the least negative d(i) among the genes called significantly repressed. The number of falsely significant genes corresponding to each permutation was computed by counting the number of genes that exceeded the horizontal cutoffs for induced and repressed genes. The estimated number of falsely significant genes was the average of the number of genes called significant from all 36 permutations. Table 1, attached hereto as appendix A and made part of this application, shows the results for different values of Δ. For Δ = 1.2, the permuted data sets generated an average of 8.4 falsely significant genes, compared to 46 genes called significant, yielding an estimated FDR of 18%. As Δ decreased, the number of genes called significant by SAM increased, but at the cost of an increasing FDR. (Omitting s$_0$ from Equation 1 produced higher FDRs of 45%, 35%, and 28% for Δ = 0.6, 0.9, and 1.2.).

Thus, as illustrated in Fig. 3A, the "observed" relative difference d(i) is plotted against expected relative difference d$_E$(i) for all of the 36 permutations. To arrive at the plot in Fig. 3A, both the "observed" and the expected relative differences are computed using the associated values of the genes in the 36 permutations using equations (1)-(3) above.

One then proceeds from the point 12 (at coordinates (0,0)) in the plot and proceed along line 14 at 45° to the axis in the positive direction along arrow 16. When the

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smallest positive "observed" relative difference $d(i)$ is encountered that exceeds the expected relative difference $d_E(i)$ by a set threshold defined by dotted line 17, such as at point 20, such value of the $d(i)$ is then set as a horizontal threshold. This value of $d(i)$ then becomes a horizontal cutoff 22, so that the number of genes with positive

5   "observed" relative difference values exceeding such threshold 22 from the 36 permutations compared to the unpermitted data would provide an indication of the false discovery rate for induced genes. This accounts for the falsely significant genes that are induced.

          To discover the falsely significant genes that are repressed, one would then

10   proceed again from point 12 along line 14 but along the negative direction 18 until one again encounters at point 30 the least negative observed relative difference $d(i)$ that exceeds the expected relative difference $d_E(i)$ by a set threshold indicated by dotted line 19. Such smallest negative $d(i)$ is then set as the negative horizontal cutoff threshold 32. The genes whose negative relative differences are more negative than such horizontal

15   cutoff 32 from the permitted and unpermitted data are used to estimate the FDR.

          To test the above described method for determining the FDR, artificial data sets are constructed in which a subset of genes was induced over a background of noise. When SAM was used to analyze such data sets, the estimated FDR accurately predicted the correct number of falsely significant genes.

20   The above method for setting thresholds provides asymmetric cutoffs for induced and repressed genes. In other words, the magnitudes of the two horizontal cutoffs 22, 32 need not be the same. The alternative is the standard t-test, which imposes a symmetric horizontal cutoff, with a $d(i) > c$ for induced genes and a $d(i) < -c$ for repressed genes. However, the asymmetric cutoff is preferred because it allows for the possibility that $d(i)$

25   for induced and repressed genes may behave differently in some biological experiments.

          Figures 4A-4C illustrate a process for comparison of SAM to conventional methods for analyzing microarrays. Figures 4A illustrate falsely significant genes plotted against number of genes called significant, where of the 57 genes most highly ranked by the fold change method, 5 were included among the 46 genes most highly ranked by

30   SAM. Of the 38 genes most highly ranked by the pairwise fold change method, 11 were included among the 46 genes most highly ranked by SAM. These results were consistent with the FDRs of SAM compared to the fold change and pairwise fold change methods.

          Figures 4B is a Northern blot validation for genes identified by the fold change method, where values of $r(i)$ are plotted for genes chosen at random from the 57 genes

most highly ranked by the fold change method. The genes are: cyclin F (1); parathymosin (2); N-acetyl glucosaminyltransferase (3); eIF-4 gamma (4); dynamin (5); interferon consensus sequence binding protein (6); heart muscle specific protein DRAL/SLIM3/FHL-2 (7), U1 snRNP-specific C protein (8); and maxi K potassium channel beta subunit (9).

5

Figure 4C is a Northern blot validation for genes identified by SAM, where results are plotted for genes chosen at random from the 46 genes most highly ranked by SAM: maxi K potassium channel beta subunit (9); cyclin B (10); PLK (11); ckshs2 (12); IL2 receptor beta chain (13); PTP(CAAX1) (14); p48 (15); XPC (16); Fas (17); and mdm2 (18).

10

SAM proved to be superior to conventional methods for analyzing microarrays (Table 1 and Figure 4A). First, SAM was compared to the approach of identifying genes as significantly changed if an R-fold change was observed. In this "fold change" method, $r(i) = \underline{x}_I(i)/\underline{x}_U(i)$, and gene (i) was called significantly changed if $r(i) > R$ or $r(i) < 1/R$. To permit computation of r(i) from negative values for gene expression, $\underline{x}_I(i)$ and $\underline{x}_U(i)$ were converted to 10 when their values were negative or less than 10. The results of this procedure yielded unacceptably high FDRs of 73% to 84%.

15

Another approach attempts to account for uncertainty in the data by identifying genes as significantly changed if an R-fold change is observed consistently between paired samples (7). To apply this "pairwise fold change" method to our 4 data sets before and 4 data sets after IR, changes in gene expression were declared significant if 12 of 16 pairings satisfied the criteria $r(i) > R$ or $r(i) < 1/R$. Despite the demand for consistent changes between paired samples, this method yielded FDRs of 60% to 71%.

20

To understand why fold-change methods fail, note that the vast majority of genes are expressed at low levels where the signal to noise ratio is very low (Figure 3C). Thus, 2-fold changes in gene expression occur at random for a large number of genes. Conversely, for higher levels of expression, smaller changes in gene expression may be real, but these changes are rejected by fold-change methods. The pairwise fold change method provides modest improvement and remained inferior to SAM.

25

Of the 46 genes most highly ranked by SAM ($\Delta = 1.2$), 36 increased or decreased at least 1.5-fold with $r(i) \geq 1.5$ or $r(i) \leq 0.67$. The number of falsely significant genes that met these two criteria was 4.5, corresponding to a FDR of 12%. Fas was identified 3 times as alternately spliced forms, leaving 34 independent genes. As an indication of biological validity, 10 of the 34 genes have been reported in the literature as part of the

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transcriptional response to IR. TNF-α was reported to be induced by others under different conditions (8) but was repressed here. We validated our microarray result by Taq-Man PCR.

To test the validity of SAM directly, Northern blots were performed for genes that were randomly selected from the 46 and 57 genes most highly ranked by SAM (Δ = 1.2) and the fold change method (at least 3.6-fold change), respectively. Northern blots showed little correlation with the genes identified by the fold change method (Figure 4B), but strong correlation with the genes identified by SAM (Figure 4C). Indeed, Northern blots contradicted only 1 of 10 genes identified by SAM, consistent with our estimated FDR.

Nineteen of the 34 genes most highly ranked by SAM appear to be involved in the cell cycle. Three are known to be induced in a p53-dependent manner: p21, cyclin G1, and mdm2 (9-11). Six cell cycle genes were repressed: ubiquitin carrier protein E2-EPF, p55cdc, cyclin B, ckshs2, cdc25 phosphatase, and wee1 kinase (12, 13). Five genes encoding the mitotic machinery were also repressed: PLK kinase, mitotic kinesin-like protein 1 (MKLP-1), mitotic centromere-associated kinesin (MCAK), cdc25 associated protein kinase (C-TAK1), and the kinetocore motor CENP-E (14-16). Four genes involved in cell proliferation were induced or repressed: the farnesylated protein tyrosine phosphatase PTP(CAAX1), OX40 ligand, lymphocyte phosphatase associated phosphoprotein (LPAP), and c-myc (17-21). Some responses were paradoxical. For example, cdc25 phosphatase and wee1 kinase have antagonistic effects on the phosphorylation state of cdc2, but both genes were repressed. Repression of these genes together with the mitotic genes may represent a damage response that dismantles the cell cycle machinery until the cell has repaired the damaged DNA.

Four of the 34 genes play roles in DNA repair, but none are involved in the repair of IR-induced double-strand breaks. Instead, the genes (p48, XPC, gadd45, PCNA) have roles in nucleotide excision repair, a pathway conventionally associated with UV-induced damage (22-25). We confirmed the induction of these genes by Northern blot (26-28). Fornace et al. reported defective removal of base damage induced by IR in xeroderma pigmentosum cells (29). Leadon et al. reported that a novel DNA repair pathway involving long excision repair patches of at least 150 nucleotides is activated by IR, but not UV (30). Our results suggest that this novel pathway might include p48, XPC, gadd45, and PCNA.

Three of the 34 genes play roles in apoptosis (Fas, bcl-2 binding component 3, TNF-α). The remaining genes may have previously unsuspected roles in the DNA damage response, or may be among the estimated set of four falsely detected genes. Attached hereto as Appendix B and made a part of this application is Table 2, which sets forth the genes with changes in expression called significant by SAM.

Discussion

The 34 genes most highly ranked by SAM are only a subset of all the genes that change 1.5-fold with IR. The difference between the number of genes called significant and the number of falsely significant genes was calculated for decreasing values of Δ = 0.3, 0.2 and 0.1, and found the difference to be 92, 170, and 184 respectively. Thus SAM suggests that at least 180 genes are induced or repressed by 5 Gy IR.

In conclusion, SAM successfully identified those genes on a microarray with *bona fide* changes in expression. Here, SAM found genes whose expression changed between two states. SAM can also be generalized to other types of experiments by expressing d(i) in other ways. Suppose the data includes gene expression $x_j(i)$ and a response parameter y_j, in which i = 1, 2, ..., m genes, j = 1, 2, ..., n samples. The generalized statistical parameter still takes the form $d(i) = r(i) / [s(i) + s_0]$. Only the definitions of r(i) and s(i) change. For example, r(i) can be correlated with factors other than irradiation, such as different type of tumors or survival time, as described in more detail below, where r(i) simply indicates relative differences in associated values, not necessarily those caused by changes due to radiation.

To identify genes whose expression is specifically different in a subset of a set of samples, the parameter d(i) is defined in terms of the Fisher's linear discriminant. One goal might be to identify genes whose expression in one type of tumor is different from its expression in other types of tumors. Suppose that a set of n samples consists of K non-overlapping subsets, with $y_j \in \{1,..., K\}$. Define $C(k) = \{j : y_j = k\}$. Let n_k = number of observations in C(k). The average gene expression in each subset is $\underline{x}_k(i) = \sum_{j \in C(k)} x_j(i)/n_k$ and the average gene expression for all n samples is $\underline{x}(i) = \sum_j x_j(i)/n$. Then define:

$$r(i) = \{[\sum_k n_k / \Pi_k n_k [\underline{x}_k(i) - \underline{x}(i)]^2\}^{1/2} \qquad (4)$$

$$s(i) = \{[\sum_k (1/n_k) / \sum_k (n_k - 1)] \sum_k \sum_{j \in C(k)} [\underline{x}_j(i) - \underline{x}_k(i)]^2\}^{1/2} \qquad (5)$$

The quantity r(i) in equation 4 is the variance between subsets, and the quantity
s(i) in equation 5 the sum of variances within each subset. Each subset may be data
5   collected from a type of tumor. Thus a large value for the generalized statistical
parameter d(i) indicates a difference in gene expression between subsets, or between the
different types of tumors. The value of s_0 in d(i) is adjusted in a manner similar to that
above by permuting the parameter k among the tumor subsets.

Thus, in general, where the associated values in each set can be classified into two
10   or more subsets with values in each subset having a correlation with one another, a
parameter may be selected using a quantity related to variances between the associated
values in the subsets of the sets and the variances of the associated values within such
subset of the sets. The quantity may relate to the sum of the variances between the
associated values and the subsets of the sets and the sum or variances of the associated
15   values within each subset of the sets.

To identify genes whose expression correlates with survival time, d(i) is defined
in terms of the Cox's proportional hazards function. Express the response data in the
form $y_j = (t_j, \delta_j)$. Here, t_j = survival time for patient (j) or censored survival time if the
patient is still alive or lost to follow-up, and $\delta_j = 0$ or 1, depending on whether patient (j)
20   was censored ($\delta_j = 0$) or died with a known survival time t_j ($\delta_j = 1$). Assume that there are
K unique death times $z_1, z_2, \ldots, z_K$. Let D(k), for k = 1, ..., K be death sets D(k) = $\{i : t_i = z_k\}$. Let R(k) be risk sets R(k) = $\{i : t_i \geq z_k\}$. Let m_k = number of patients in R(k). Let d_k =
number of deaths at time z_k. The average expression of gene (i) in death set D(k) is: $x\bar{}_k(i)$
= $\sum_{j \in D(k)} x_j(i)/d_k$. The average expression of gene (i) in risk set R(k) is: $\underline{x}_k(i) = \sum_{j \in R(k)}$
25     $x_j(i)/m_k$. Then define:

$$r(i) = \sum_k d_k [x\bar{}_k(i) - \underline{x}_k(i)] \qquad (6)$$

30     $$s(i) = \{\sum_k (d_k/m_k)\sum_{j \in R(k)} [x_j(i) - \underline{x}_k(i)]^2\}^{1/2} \qquad (7)$$

SAM can be adapted for still other types of experimental data. For example, to
identify genes whose expression correlates with a quantitative parameter, such as tumor

stage, d(i) can be defined in terms of the Pearson correlation coefficient, as described in more detail in the example below.

A method for identifying genes whose expression correlates with a continuous parameter would be one identifying genes whose expression in a tumor correlates with survival time of the patient with the tumor.

Let $x_k(i)$ be the expression of gene (i) in sample (k) (e.g., the kth tumor). Define $\underline{x}(i)$ to be the average expression of gene (i) over all the samples.

Let y_k be the value for the continuous parameter (e.g., time) associated with sample (k). Define $\underline{y}$ to be the average of the continuous parameter over all the samples.

The Pearson correlation coefficient, r(i) for gene (i) is:

$$r(i) = \sum{}_k [x_k(i) - \underline{x}(i)] \, [y_k - \underline{y}] \Big/ [\sum{}_k [x_k(i) - \underline{x}(i)]^2 \sum{}_k (y_k - \underline{y})^2]^{1/2} \qquad (8)$$

The values for r(i) are less than +1 and greater than −1. For r(i) ≈ +1, the corrleation is strongly positive. For r(i) ≈ −1, the correlation is strongly negative. An example of a modified Pearson correlation coefficient that could serve as the parameter d(i) is:

$$d(i) = \sum{}_k [x_k(i) - \underline{x}(i)][y_k - \underline{y}] \Big/ \{[\sum{}_k [x_k(i) - \underline{x}(i)]^2 \sum{}_k (y_k - \underline{y})^2]^{1/2} + s_o\} \qquad (9)$$

The value of s_0 would be adjusted in the manner described above, thus permitting comparison across the entire set of genes. To compute the expected d(i), the survival times are permuted among the tumors.

In addition to applications using the Pearson correlation coefficient, another example includes the definition of d(i) for paired data, such as gene expression in tumors before and after chemotherapy. In each case, the FDR is estimated by random permutation of the data for gene expression among the different experimental arms, i.e., permutations among the n arms of y_j.

Weighting Function to Improve Data Reproducibility

For microarrays that contain several probes for each gene, expression is typically computed as a simple mean or a trimmed mean, which eliminates

contributions from probes that diverge from the mean by a predetermined cutoff. Such methods fail to eliminate uncertainty in the data arising from probes that do not behave appropriately as shown in Fig. 5A.

Data reproducibility can be improved by modifying the contribution of each probe by a continuous weighting function. For example, the weight for probe (i) of a given gene can be determined by a Guassian weight function,

$$w(i) = \exp\left[-\left(x_1 - x_0\right)^2 / a^2\right] \qquad (10)$$

where x_0 = mean or median of data from all the probes for the gene, a = constant multiplied by standard deviation or median absolute deviation of data. When the Gaussian weight function was applied to an experiment in which the same sample was hybridized twice to two microarrays, there was major improvement in the data (Fig. 5B). The scatter function decreased by more than a factor of two and the number of negatively expressed genes decreased from 25% to 0.001%.

Thus, SAM is a robust and straightforward method that can be adapted to a broad range of experimental situations. SAM and its modifications are available for use at http://www-stat-class.stanford.edu/SAM/SAMServlet. This web site is used at Stanford University.

Software Implementation

The invention has been described above, employing methods and producing plots as illustrated in the Figures. Such methods and graphs or plots may be produced with the aid of machines such as computers. Therefore, another aspect of the invention involves the software components that are loaded to a computer to perform the above-described functions. These functions provide results with the different advantages outlined above. The software or program components may be installed in a computer in a variety of ways.

As will be understood in the art, the inventive software components may be embodied in a fixed media program component containing logic instructions and/or data that when loaded into an appropriately configured computing device to cause that device to perform according to the invention. As will be understood in the art, a fixed media program may be delivered to a user on a fixed media for loading in a users

computer or a fixed media program can reside on a remote server that a user accesses through a communication medium in order to download a program component. Thus another aspect of the invention involves transmitting, or causing to be transmitted, the program component to a user where the component, when downloaded into the user's

5   device, can perform any one or more of the functions described above.

Fig. 6 shows an information appliance (or digital device) 40 that may be understood as a logical apparatus that can read instructions from media 47 and/or network port 49. Apparatus 40 can thereafter use those instructions to direct server or client logic, as understood in the art, to embody aspects of the invention. One type of

10 logical apparatus that may embody the invention is a computer system as illustrated in 40, containing CPU 44, optional input devices 49 and 41, disk drives 45 and optional monitor 46. Fixed media 47 may be used to program such a system and may represent a disk-type optical or magnetic media, magnetic tape, solid state memory, etc.. One or more aspects of the invention may be embodied in whole or in part as

15 software recorded on this fixed media. Communication port 49 may also be used to initially receive instructions that are used to program such a system to perform any one or more of the above-described functions and may represent any type of communication connection, such as to the internet or any other computer network. The instructions or program may be transmitted directly to a user's device or be

20 placed on a network, such as a website of the internet to be accessible through a user's device. All such methods of making the program or software component available to users are known to those in the art and will not be described here.

The invention also may be embodied in whole or in part within the circuitry of an application specific integrated circuit (ASIC) or a programmable logic device

25 (PLD). In such a case, the invention may be embodied in a computer understandable descriptor language which may be used to create an ASIC or PLD that operates as herein described.

While the invention has been described above by reference to various embodiments, it will be understood that changes and modifications may be made

30 without departing from the scope of the invention, which is to be defined only by the appended claims and their equivalents. All references referred to herein are incorporated by reference in their entireties.

APPENDIX A

Table 1. Comparison of methods for identifying changes in gene expression. To increase the stringency for calling significant changes in gene expression, parameters for each method (Δ and R) were increased, as described in the text. The number of falsely significant genes was estimated by permutation of the data sets. The false discovery rate (FDR) was defined as the percentage of falsely significant genes compared to the genes called significant.

Method	Genes falsely significant	Genes called significant	FDR
SAM			
$\Delta = 0.4$	134.9	288	47%
$\Delta = 0.5$	78.1	192	41%
$\Delta = 0.6$	56.1	162	35%
$\Delta = 0.9$	19.1	80	24%
$\Delta = 1.2$	8.4	46	18%
Fold change			
R = 2.0	283.1	348	81%
R = 2.5	137.8	169	82%
R = 3.0	76.8	99	78%
R = 3.5	46.7	64	73%
R = 4.0	29.3	35	84%
Pairwise fold change			
R = 1.2	245.6	355	69%
R = 1.3	155.4	220	71%
R = 1.5	76.2	118	65%
R = 1.7	44.8	70	64%
R = 2.0	22.8	38	60%

APPENDIX B

Table 2. Genes with changes in expression called significant by SAM.

* To compute $r(i) = x_I(i)/x_U(i)$, negative levels of expression were reset to a value of 10.

† Genes previously reported to respond transcriptionally to ionizing radiation.

Gene functions: Black = cell cycle; Dark gray = apoptosis; Light gray = DNA repair

Rank	Accession	d(i)	r(i)	s(i)	$x_U(i)$	$x_I(i)$	Gene
INDUCED GENES							
1	U09579 †	9.2	3.4	158	633	2119	p21
2	X83490 †	7.7	2.5	26	155	381	Fas (alternate splice deleting exons 3 & 4)
3	U47621	7.1	2.9	13	61	178	No55 nucleolar autoantigen
4	U18300 †	6.9	1.9	59	448	869	p48, xeroderma pigmentosum group E gen
5	U48296	6.7	1.6	30	354	583	protein tyrosine phosphatase PTP(CAAX1
6	X63717 †	6.6	2.2	43	254	561	Fas
7	D21089	5.5	2.4	96	392	930	XPC, xeroderma pigmentosum group C ge
8	U39400	5.4	1.7	41	349	581	NOF1
9	X77794 †	5.3	1.6	99	964	1499	cyclin G1
10	M60974 †	5.1	2.5	58	203	516	gadd45
11	D90224	4.9	2.8	32	96	270	OX40 ligand, TNF ligand superfamily
12	U25138	4.9	9.0*	16	-4	90	maxi K potassium channel beta subunit
13	J05614 †	4.8	1.7	352	2358	4043	PCNA, proliferating cell nuclear antigen
14	X83492 †	4.8	1.8	17	117	213	Fas (alternate splice deleting exons 4 & 7)
15	X85116	4.5	2.0	15	83	163	EPB72, integral membrane protein
16	U50136	4.4	1.6	26	231	359	leukotriene C4 synthase (LTC4S)
17	U82987	4.2	33.6*	161	-4	336	bcl-2 binding component 3 (bbc3)
18	M92424 †	4.0	2.8	16	45	125	mdm2
REPRESSED GENES							
1	U01038	-9.8	0.50	25	551	275	PLK, polo kinase homolog
2	M91670	-9.3	0.61	25	693	425	ubiquitin carrier protein (E2-EPF)
3	U68233	-9.3	0.48	12	275	133	HRR-1 farnesol receptor
4	U05340	-6.9	0.39	54	642	253	p55cdc; present in dividing cells
5	M25753 †	-5.8	0.23	43	345	78	cyclin B
6	X97267	-5.8	0.49	69	818	400	lymphocyte phosphatase assoc phosphopr
7	S78187 †	-5.5	0.63	20	357	224	cdc25 phosphatase
8	X54942	-5.5	0.52	87	1026	534	ckshs2, cks1 protein homolog
9	U63743	-5.0	0.59	19	265	158	MCAK, mitotic centromere-associated kin
10	D86973	-4.9	0.64	16	264	168	GCN1, translational regulator of GCN4
11	X62048 †	-4.9	0.44	8	99	43	wee1 kinase
12	M80359	-4.9	0.40*	6	25	-19	C-TAK1, cdc25c associated protein kinase
13	U28386	-4.8	0.58	77	928	541	hSRP1α, NLS receptor
14	X02910	-4.8	0.37	20	170	63	tumor necrosis factor (TNF-α)
15	D31764	-4.7	0.26	36	247	64	hEphB1b, Eph-like receptor tyrosine kinas
16	X67155	-4.4	0.30	28	199	60	MKLP-1, mitotic kinesin-like protein-1
17	HG3523	-4.4	0.51	40	391	200	c-Myc, alternate splice form 3
18	Z15005	-4.2	0.28*	10	36	-20	CENP-E putative kinetochore motor

WHAT IS CLAIMED IS:

1. A method for analyzing a plurality of sets of values associated with a plurality of genes to identify genes whose associated values differ by an amount of statistical significance among the sets, wherein each of the sets of associated values of the genes is obtained from one of a number of data sources, wherein the method comprises:

    providing for each of the plurality of genes a parameter that contains information concerning differences in the associated values of that gene among the sets;

    adjusting the parameters of the plurality of genes so that the parameters are substantially independent of scatter values or average associated values of the genes over the sets;

    deriving an observed value and an expected value of the adjusted parameter for each gene from the sets of associated values; and

    comparing the observed and expected values of the parameter to identify genes whose associated values differ by an amount of statistical significance among the sets.

2. The method of claim 1, wherein said adjusting includes:

    dividing the scatter values or average associated values of the genes into subsets each having a similar range of values, and calculating the standard deviation of each of the parameters within each subset;

    altering the parameters until a coefficient of variation of the standard deviations of the parameters among the subsets is minimized.

3. The method of claim 1, further comprising obtaining said sets of associated values from multiple measurements of the plurality of genes, or values derived therefrom.

4. The method of claim 1, wherein said sets of associated values represent gene expression or number of gene copies or levels of protein encoded by the genes.

5. The method of claim 1, wherein said sets of associated values include calculated or predicted values.

6.   The method of claim 1, wherein said providing includes calculating a
difference value between an associated value of each gene in a first of the sets or a
value derived therefrom and an associated value of that gene in a second of the sets or
a value derived therefrom; wherein the parameter is a function of the difference value
5   of that gene.

7.   The method of claim 6, wherein said providing further includes:
generating for each of the plurality of genes a scatter value that quantifies variation in
the associated values of that gene within the first and second sets; and wherein said
parameter is a function of the scatter value and of the difference value, said parameter
10   defining a relative difference value of that gene.

8.   The method of claim 7, wherein said generating employs the following
equation:

$$s(i) = (\{1/a\}\{\sum{}_m[x_m(i) - \underline{x}_I(i)]^2 + \sum{}_n[x_n(i) - \underline{x}_U(i)]^2\})^{1/2}$$

where gene *(i)* has associated values $x_I(i)$ and $x_U(i)$ in Ith and Uth states respectively in
15   the first and second sets of associated values, I and U being positive integers; $\sum{}_m$ and
$\sum{}_n$ are sums over associated values of gene *(i)* in states I in the first set and in states U
in the second set respectively, where *s(i)* is the scatter value of gene *(i)*, and a is a
constant.

9.   The method of claim 8, wherein said calculating calculates the
20   parameter d(i) from the following equation:

$$d(i) = [\underline{x}_I(i) - \underline{x}_U(i)]/[s(i) + s_o]$$

where s_0 is a constant, and $\underline{x}_I(i)$ and $\underline{x}_U(i)$ are the average values of $x_I(i)$ and $x_U(i)$
respectively in the first and second sets of associated values.

25   10.   The method of claim 9, further comprising:
dividing the scatter values or average associated values of the genes into
subsets each having a similar range of values, and calculating the standard deviation
of each of the parameters within each subset; and
altering value of s_0 until a coefficient of variation of the standard deviations of
30   the parameters among the subsets is minimized.

11.   The method of claim 1, wherein said associated values of the genes are correlated with another variable so that each of said associated values has a corresponding value of the variable, and wherein the parameter is provided using a Pearson correlation coefficient related to a weighted difference between each of the

5   associated values and an average associated value, the variance of the associated values and the variance of the variable, said difference weighted by deviation of the corresponding value of the variable of such associated value from its average value.

12.   The method of claim 11, wherein said variable is continuous.

13.   The method of claim 12, wherein said variable is time.

10   14.   The method of claim 11, wherein the parameter is selected using the Pearson correlation coefficient and a quantity s_0 that has a value adjusted as follows:

dividing the scatter values or average associated values of the genes into subsets each having a similar range of values, and calculating the standard deviation of each of the parameters within each subset; and

15   altering value of s_0 until a coefficient of variation of the standard deviations of the parameters among the subsets is minimized.

15.   The method of claim 11, the number of sets of associated values being k, k being a positive integer, wherein said Pearson correlation coefficient r(i) is given by:

20   $$r(i) = \sum_{k}[(x_k(i) - \underline{x}(i))][(y_k - \underline{y})] / \sqrt{\sum_{k}(x_k(i) - \underline{x}(i))^2 \sum_{k}(y_k - \underline{y})^2}$$

where $x_k(i)$ is the associated value of gene (i) in the kth set of associated values, $\underline{x}(i)$ the average of the associated values of gene (i) in all the sets, y_k the value of the variable corresponding to $x_k(i)$, $\underline{y}$ the average value of y_k in all the sets; and $\sum_k$ is a sum over all values of k.

25   16.   The method of claim 1, wherein the associated values in each set are classified into two or more subsets with values in each subset having a correlation with one another, and wherein the parameter is selected using a quantity related to variances between the associated values in the subsets of the sets and the variances of the associated values within each subset of the sets.

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17.   The method of claim 16, wherein the quantity relates to the sum of variances between the associated values in the subsets of the sets and the sum of variances of the associated values within each subset of the sets.

18.   The method of claim 17, wherein the parameter is selected using the
5   Fisher discriminant and a quantity s_0 having a value which has been adjusted as follows:

dividing the scatter values or average associated values of the genes into subsets each having a similar range of values, and calculating the standard deviation of each of the parameters within each subset; and

10   altering value of s_0 until a coefficient of variation of the standard deviations of the parameters among the subsets is minimized.

19.   The method of claim 18, wherein the number of subsets of associated values of such set being k, k being a positive integer, and the Fisher discriminant F(i) is given by:

15   $$F(i) = \sum_k n_k [x_k(i) - \underline{x}(i)]^2 / \sum_k \sum_j [x_j(i) - \underline{x}_k(i)]^2$$

where $x_k(i)$ is an associated value of gene (i) in the kth subset of associated values, $\underline{x}_k(i)$ the average of the associated values of gene (i) in the kth subset, $\underline{x}(i)$ the average value of the associated values of gene (i) in all of the subsets, n_k the number of associated values in the kth set, $\sum_j$ a sum over all the associated values of gene (i) in
20   the kth subset, and $\sum_k$ a sum of the associated values of gene (i) over all of the subsets.

20.   The method of claim 1, the sets of associated values referred to as original sets, wherein said deriving includes deriving said expected value by:

permuting, for each of the plurality of genes, the associated values for such gene in the original sets to arrive at a number of different permutations;
25   classifying the associated values in each permutation of each gene into corresponding permuted sets that are different from the original sets; and

supplying for each permutation a parameter value of each of the genes derived from an associated value of such gene in each of the corresponding permuted sets for such permutation or values derived therefrom.

21.   The method of claim 20, wherein said associated values of the genes are correlated with another variable so that each of said associated values has an associated value of the variable, wherein the permuting permutes the associated values so that at least each of some of the associated values has a different associated

5   variable.

22.   The method of claim 21, wherein the associated values are classified into two or more subsets with values in each subset having a correlation with one another, wherein the permuting permutes the associated values so that at least each of some of the associated values is in a subset different from the subset it is classified

10   into.

23.   A method for analyzing a plurality of sets of values associated with a plurality of genes to identify genes whose associated values differ by an amount of statistical significance among the sets, wherein the associated values correlate with patient survival time, and wherein the associated values of the genes are obtained

15   from a number of data sources, said method comprising:

defining pairs of death and risk sets, each pair having a corresponding patient death time, where the death set of such pair includes associated values corresponding to the death time of such pair and the risk set of such pair includes associated values corresponding to times occurring after the death time of such pair;

20   providing for each of the plurality of genes a parameter that contains information concerning differences in the associated values of that gene among the sets;

deriving an observed value and an expected value of the parameter for each gene from the sets of associated values; and

25   comparing the observed and expected values of the parameter to identify genes whose associated values differ by an amount of statistical significance.

24.   The method of claim 23, wherein said providing provides said parameter as a function of weighted differences between the average associated values of the death and risk sets of the pairs, and of weighted variances within the risk

30   sets.

25.   The method of claim 24, wherein said providing provides for gene *(i)* said parameter by means of r(i) and s(i) given by the following:

$$r(i) = \sum_k d_k [x^*_k(i) - \underline{x}_k(i)]$$

$$s(i) = \{\sum_k (d_k/m_k) \sum_{j \in R(k)} [x_j(i) - \underline{x}_k(i)]^2\}^{1/2}$$

where there are K unique death times $z_1, z_2, \ldots, z_K$;

D(k), for k = 1, ..., K, are death sets defined by $D(k) = \{i : t_i = z_k\}$;

R(k) are risk sets defined by $R(k) = \{i : t_i \geq z_k\}$;

m_k is number of patients in R(k);

d_k is number of patient deaths at time z_k;

an average expression of gene (i) in death set D(k) is given by:

$x^*_k(i) = \sum_{j \in D(k)} x_j(i)/d_k$; and

an average expression of gene (i) in risk set R(k) is given by:

$\underline{x}_k(i) = \sum_{j \in R(k)} x_j(i)/m_k$.

26.   The method of claim 24, wherein said providing provides said parameter by means of r(i) and s(i) given by the following: $r(i)/[s(i)+s_0]$, where s_0 is a constant.

27.   The method of claim 24, further comprising:

dividing the scatter values or average associated values of the genes into subsets each having a similar range of values, and calculating the standard deviation of each of the parameters within each subset; and

altering value of s_0 until a coefficient of variation of the standard deviations of the parameters among the subsets is minimized.

28.   A method for analyzing a plurality of original sets of values associated with a plurality of genes to identify genes whose associated values differ by an amount of statistical significance among the sets, wherein each of the sets of associated values of the genes is obtained from one of a number of data sources, wherein the method comprises:

calculating for each gene a value for a statistical parameter indicating differences between associated values of such gene among the original sets;

ranking the values of the parameter of the genes;

providing an expected value of such parameter for each rank, wherein said providing includes permuting the associated values in the original sets to arrive at sets different from the original sets for each permutation, deriving a value of such parameter for each permutation, and ranking such values; and

5   comparing the calculated and expected values for the parameter of the same rank to identify genes whose associated values differ by an amount of statistical significance among the sets.

29.   The method of claim 28, wherein said providing comprises:

10   for each permutation, deriving a value of the parameter for each gene and ranking the genes by their associated parameter values; and

determining the expected value of such parameter for each rank by computing an average value of the parameter of all the permutations having such rank.

15   30.   The method of claim 29, wherein said comparing comprises identifying a gene as one whose associated values differ by an amount of statistical significance among the sets when the difference for such gene between the calculated value of the parameter of a rank and the expected value of such parameter of the same rank exceeds a threshold.

20

31.   The method of claim 29, wherein said method further comprises identifying a lowest rank gene whose parameter value derived for a permutation is positive and exceeds a first threshold, setting such parameter value as a second threshold, comparing the derived parameter values of other genes for permutations to

25   the second threshold and calling each gene whose derived parameter value exceeds the second threshold as a gene whose associated values are falsely identified to differ by an amount of statistical significance among the sets.

32.   The method of claim 29, wherein said method further comprises identifying a lowest rank gene whose parameter value derived for a permutation is

30   negative and less than a first threshold, setting such parameter value as a second threshold, comparing the derived parameter values of other genes for permutations to the second threshold and calling each gene whose derived parameter value is less than

the second threshold as a gene whose associated values are falsely identified to differ by an amount of statistical significance among the sets.

33. The method of claim 28, wherein the sets of associated values in each permutation contains approximately an equal number of associated values from each 5 of the original sets of associated values.

34. A method for analyzing a plurality of original sets of values associated with a plurality of genes to identify genes whose associated values are falsely identified to differ by an amount of statistical significance among the sets, wherein each of the sets of associated values of the genes is obtained from one of a number of 10 data sources, wherein the method comprises:

defining for each gene a statistical parameter indicating differences between associated values of such gene among the original sets;

providing an expected value of such parameter for each gene, wherein said providing includes permuting the associated values in the sets to arrive at sets 15 different from the original sets for each permutation, deriving a value of such parameter for each permutation, and ranking such values;

deriving for each gene a value for the parameter for each permutation and ranking the genes by their derived parameter values;

finding a lowest rank gene whose derived parameter value extends beyond a 20 first threshold; and

comparing the derived parameter values of other genes for permutations to the second threshold and calling each gene whose derived parameter value extends beyond the second threshold as a gene whose associated values are falsely identified to differ by an amount of statistical significance among the sets.

25 35. A method for reducing statistical error of a set of associated values of genes, wherein the method comprises:

providing a set of associated values of each gene; and

processing said set of associated values of that gene using a smooth weighting function to yield a representative value for that gene.

30 36. The method of claim 35, wherein said processing uses a Gaussian weighting function.

37.   A method for comparing sets of associated values of genes, which comprises:

providing sets of associated values of each gene;

5   processing said sets of associated values of that gene using a smooth weighting function to obtain a representative value for that gene from each of the sets; and

comparing representative values for that gene for the sets.

38.   The method of claim 37, wherein said providing includes calculating a difference PM-MM of a probe pair of a microarray.

10

39.   A method for comparing a first and a second set of associated values of genes, which comprises:

providing odd root values of the values in the first set, and odd root values of the values in the second set; and

15   comparing the odd root values of the values in the first set and the odd root values of the values in the second sets.

40.   The method of claim 39, wherein said providing provides the cube or fifth root values of the values in the first or second sets.

41.   The method of claim 40, wherein said representing includes scaling the
20   odd root values along the two axes, and wherein said method further comprises providing a best fit curve for the odd root values of the first and second set in the plot.

42.   The method of claim 39, wherein said comparing includes representing the odd root values of the values in the first set along a first axis of a two-dimensional plot and the odd root values of the values in the second set along a second axis of the
25   plot.

43.   The method of claim 39, wherein said odd root values provided and compared includes values derived from positive and negative associated values.

44.   A computer readable storage device embodying a program of instructions executable by a computer to perform a method for analyzing a plurality of
30   sets of values associated with a plurality of genes to identify genes whose associated

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values differ by an amount of statistical significance among the sets, wherein each of the sets of associated values of the genes is obtained from one of a number of data sources, wherein the method comprises:

5   providing for each of the plurality of genes a parameter that contains information concerning differences in the associated values of that gene among the sets;

adjusting the parameters of the plurality of genes so that the parameters are substantially independent of scatter values or average associated values of the genes over the sets;

10   deriving an observed value and an expected value of the adjusted parameter for each gene from the sets of associated values; and

comparing the observed and expected values of the parameter to identify genes whose associated values differ by an amount of statistical significance among the sets.

15

45.   A computer readable storage device embodying a program of instructions executable by a computer to perform a method for analyzing a plurality of sets of values associated with a plurality of genes to identify genes whose associated values differ by an amount of statistical significance among the sets, wherein the

20   associated values correlate with patient survival time, and wherein the associated values of the genes are obtained from a number of data sources, said method comprising:

defining pairs of death and risk sets, each pair having a corresponding patient death time, where the death set of such pair includes associated values corresponding

25   to the death time of such pair and the risk set of such pair includes associated values corresponding to times occurring after the death time of such pair;

providing for each of the plurality of genes a parameter that contains information concerning differences in the associated values of that gene among the sets;

30   deriving an observed value and an expected value of the parameter for each gene from the sets of associated values; and

comparing the observed and expected values of the parameter to identify genes whose associated values differ by an amount of statistical significance.

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46.   A computer readable storage device embodying a program of instructions executable by a computer to perform a method for analyzing a plurality of original sets of values associated with a plurality of genes to identify genes whose associated values differ by an amount of statistical significance among the sets,

5   wherein each of the sets of associated values of the genes is obtained from one of a number of data sources, wherein the method comprises:

calculating for each gene a value for a statistical parameter indicating differences between associated values of such gene among the original sets;

ranking the values of the parameter of the genes;

10   providing an expected value of such parameter for each rank, wherein said providing includes permuting the associated values in the original sets to arrive at sets different from the original sets for each permutation, deriving a value of such parameter for each permutation, and ranking such values; and

comparing the calculated and expected values for the parameter of the same

15   rank to identify genes whose associated values differ by an amount of statistical significance among the sets.

47.   A computer readable storage device embodying a program of instructions executable by a computer to perform a method for analyzing a plurality of

20   original sets of values associated with a plurality of genes to identify genes whose associated values are falsely identified to differ by an amount of statistical significance among the sets, wherein each of the sets of associated values of the genes is obtained from one of a number of data sources, wherein the method comprises:

defining for each gene a statistical parameter indicating differences between

25   associated values of such gene among the original sets;

providing an expected value of such parameter for each gene, wherein said providing includes permuting the associated values in the sets to arrive at sets different from the original sets for each permutation, deriving a value of such parameter for each permutation, and ranking such values;

30   deriving for each gene a value for the parameter for each permutation and ranking the genes by their derived parameter values;

finding a lowest rank gene whose derived parameter value extends beyond a first threshold; and

comparing the derived parameter values of other genes for permutations to the second threshold and calling each gene whose derived parameter value extends beyond the second threshold as a gene whose associated values are falsely identified to differ by an amount of statistical significance among the sets.

5   48.   A computer readable storage device embodying a program of instructions executable by a computer to perform a method for reducing statistical error of a set of associated values of genes, wherein the method comprises:

providing a set of associated values of each gene; and

processing said set of associated values of that gene using a smooth weighting
10   function to yield a representative value for that gene.

49.   A computer readable storage device embodying a program of instructions executable by a computer to perform a method for comparing sets of associated values of genes, which comprises:

providing sets of associated values of each gene;
15   processing said sets of associated values of that gene using a smooth weighting function to obtain a representative value for that gene from each of the sets; and

comparing representative values for that gene for the sets.

50.   A computer readable storage device embodying a program of
20   instructions executable by a computer to perform a method for comparing a first and a second set of associated values of genes, which comprises:

providing odd root values of the values in the first set, and odd root values of the values in the second set; and

comparing the odd root values of the values in the first set and the odd root
25   values of the values in the second sets.

51.   A method for transmitting a program of instructions executable by a computer to perform a method for analyzing a plurality of sets of values associated with a plurality of genes to identify genes whose associated values differ by an amount of statistical significance among the sets, wherein each of the sets of
30   associated values of the genes is obtained from one of a number of data sources, wherein the method comprises:

causing a program of instructions to be transmitted to a client device, thereby enabling the client device to perform, by means of such program, the following process:

providing for each of the plurality of genes a parameter that contains

5   information concerning differences in the associated values of that gene among the sets;

adjusting the parameters of the plurality of genes so that the parameters are substantially independent of scatter values or average associated values of the genes over the sets;

10   deriving an observed value and an expected value of the adjusted parameter for each gene from the sets of associated values; and

comparing the observed and expected values of the parameter to identify genes whose associated values differ by an amount of statistical significance among the sets.

15

52.   A method for transmitting a program of instructions executable by a computer to perform a method for analyzing a plurality of sets of values associated with a plurality of genes to identify genes whose associated values differ by an amount of statistical significance among the sets, wherein the associated values

20   correlate with patient survival time, and wherein the associated values of the genes are obtained from a number of data sources, said method comprising:

causing a program of instructions to be transmitted to a client device, thereby enabling the client device to perform, by means of such program, the following process:

25   defining pairs of death and risk sets, each pair having a corresponding patient death time, where the death set of such pair includes associated values corresponding to the death time of such pair and the risk set of such pair includes associated values corresponding to times occurring after the death time of such pair;

providing for each of the plurality of genes a parameter that contains

30   information concerning differences in the associated values of that gene among the sets;

deriving an observed value and an expected value of the parameter for each gene from the sets of associated values; and

comparing the observed and expected values of the parameter to identify genes whose associated values differ by an amount of statistical significance.

53.   A method for transmitting a program of instructions executable by a computer to perform a method for analyzing a plurality of original sets of values

5   associated with a plurality of genes to identify genes whose associated values differ by an amount of statistical significance among the sets, wherein each of the sets of associated values of the genes is obtained from one of a number of data sources, wherein the method comprises:

            causing a program of instructions to be transmitted to a client device, thereby

10   enabling the client device to perform, by means of such program, the following process:

            calculating for each gene a value for a statistical parameter indicating differences between associated values of such gene among the original sets;

            ranking the values of the parameter of the genes;

15   providing an expected value of such parameter for each rank, wherein said providing includes permuting the associated values in the original sets to arrive at sets different from the original sets for each permutation, deriving a value of such parameter for each permutation, and ranking such values; and

            comparing the calculated and expected values for the parameter of the same

20   rank to identify genes whose associated values differ by an amount of statistical significance among the sets.

54.   A method for transmitting a program of instructions executable by a computer to perform a method for analyzing a plurality of original sets of values

25   associated with a plurality of genes to identify genes whose associated values are falsely identified to differ by an amount of statistical significance among the sets, wherein each of the sets of associated values of the genes is obtained from one of a number of data sources, wherein the method comprises:

            causing a program of instructions to be transmitted to a client device, thereby

30   enabling the client device to perform, by means of such program, the following process:

            defining for each gene a statistical parameter indicating differences between associated values of such gene among the original sets;

providing an expected value of such parameter for each gene, wherein said providing includes permuting the associated values in the sets to arrive at sets different from the original sets for each permutation, deriving a value of such parameter for each permutation, and ranking such values;

5   deriving for each gene a value for the parameter for each permutation and ranking the genes by their derived parameter values;

finding a lowest rank gene whose derived parameter value extends beyond a first threshold; and

comparing the derived parameter values of other genes for permutations to the
10   second threshold and calling each gene whose derived parameter value extends beyond the second threshold as a gene whose associated values are falsely identified to differ by an amount of statistical significance among the sets.

55.   A method for transmitting a program of instructions executable by a computer to perform a method for reducing statistical error of a set of associated
15   values of genes, wherein the method comprises:

causing a program of instructions to be transmitted to a client device, thereby enabling the client device to perform, by means of such program, the following process:

providing a set of associated values of each gene; and

20   processing said set of associated values of that gene using a smooth weighting function to yield a representative value for that gene.

56.   A method for transmitting a program of instructions executable by a computer to perform a method for comparing sets of associated values of genes, which comprises:

25   causing a program of instructions to be transmitted to a client device, thereby enabling the client device to perform, by means of such program, the following process:

providing sets of associated values of each gene;

processing said sets of associated values of that gene using a smooth
30   weighting function to obtain a representative value for that gene from each of the sets; and

comparing representative values for that gene for the sets.

57.   A method for transmitting a program of instructions executable by a computer to perform a method for comparing a first and a second set of associated values of genes, which comprises:

    causing a program of instructions to be transmitted to a client device, thereby

5   enabling the client device to perform, by means of such program, the following process:

    providing odd root values of the values in the first set, and odd root values of the values in the second set; and

    comparing the odd root values of the values in the first set and the odd root

10   values of the values in the second sets.

58.   A computer system for analyzing a plurality of sets of values associated with a plurality of genes to identify genes whose associated values differ by an amount of statistical significance among the sets, wherein each of the sets of associated values of the genes is obtained from one of a number of data sources,

15   wherein the system comprises:

    one or more computers;

    one or more computer programs running on the computer(s), performing the following:

    providing for each of the plurality of genes a parameter that contains

20   information concerning differences in the associated values of that gene among the sets;

    adjusting the parameters of the plurality of genes so that the parameters are substantially independent of scatter values or average associated values of the genes over the sets;

25   deriving an observed value and an expected value of the adjusted parameter for each gene from the sets of associated values; and

    comparing the observed and expected values of the parameter to identify genes whose associated values differ by an amount of statistical significance among the sets.

30

59.   A computer system for analyzing a plurality of sets of values associated with a plurality of genes to identify genes whose associated values differ by an amount of statistical significance among the sets, wherein the associated values

correlate with patient survival time, and wherein the associated values of the genes are obtained from a number of data sources, said system comprising:

one or more computers;

one or more computer programs running on the computer(s), performing the

5   following:

defining pairs of death and risk sets, each pair having a corresponding patient death time, where the death set of such pair includes associated values corresponding to the death time of such pair and the risk set of such pair includes associated values corresponding to times occurring after the death time of such pair;

10   providing for each of the plurality of genes a parameter that contains information concerning differences in the associated values of that gene among the sets;

deriving an observed value and an expected value of the parameter for each gene from the sets of associated values; and

15   comparing the observed and expected values of the parameter to identify genes whose associated values differ by an amount of statistical significance.

60.   A computer system for analyzing a plurality of original sets of values associated with a plurality of genes to identify genes whose associated values differ by an amount of statistical significance among the sets, wherein each of the sets of

20   associated values of the genes is obtained from one of a number of data sources, wherein the system comprises:

one or more computers;

one or more computer programs running on the computer(s), performing the following:

25   calculating for each gene a value for a statistical parameter indicating differences between associated values of such gene among the original sets;

ranking the values of the parameter of the genes;

providing an expected value of such parameter for each rank, wherein said providing includes permuting the associated values in the original sets to arrive at sets

30   different from the original sets for each permutation, deriving a value of such parameter for each permutation, and ranking such values; and

comparing the calculated and expected values for the parameter of the same rank to identify genes whose associated values differ by an amount of statistical significance among the sets.

5   61.   A computer system for analyzing a plurality of original sets of values associated with a plurality of genes to identify genes whose associated values are falsely identified to differ by an amount of statistical significance among the sets, wherein each of the sets of associated values of the genes is obtained from one of a number of data sources, wherein the system comprises:

10   one or more computers;

one or more computer programs running on the computer(s), performing the following:

defining for each gene a statistical parameter indicating differences between associated values of such gene among the original sets;

15   providing an expected value of such parameter for each gene, wherein said providing includes permuting the associated values in the sets to arrive at sets different from the original sets for each permutation, deriving a value of such parameter for each permutation, and ranking such values;

deriving for each gene a value for the parameter for each permutation and 20 ranking the genes by their derived parameter values;

finding a lowest rank gene whose derived parameter value extends beyond a first threshold; and

comparing the derived parameter values of other genes for permutations to the second threshold and calling each gene whose derived parameter value extends 25 beyond the second threshold as a gene whose associated values are falsely identified to differ by an amount of statistical significance among the sets.

62.   A computer system for reducing statistical error of a set of associated values of genes, wherein the system comprises:

one or more computers;

30   one or more computer programs running on the computer(s), performing the following:

providing a set of associated values of each gene; and

processing said set of associated values of that gene using a smooth weighting function to yield a representative value for that gene.

63. A computer system for comparing sets of associated values of genes, which comprises:

5   one or more computers;

one or more computer programs running on the computer(s), performing the following:

providing sets of associated values of each gene;

processing said sets of associated values of that gene using a smooth

10 weighting function to obtain a representative value for that gene from each of the sets; and

comparing representative values for that gene for the sets.

64. A computer system for comparing a first and a second set of associated

15 values of genes comprising

one or more computers;

one or more computer programs running on the computer(s), performing the following:

providing odd root values of the values in the first set, and odd root values of

20 the values in the second set; and

comparing the odd root values of the values in the first set and the odd root values of the values in the second sets.

25
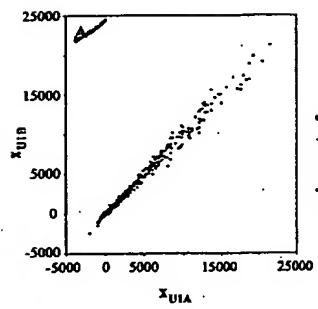
FIG. 1A   FIG. 1B
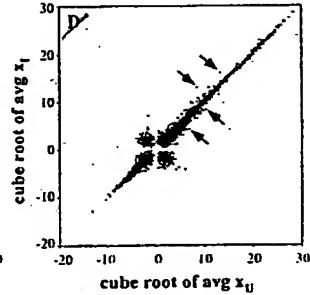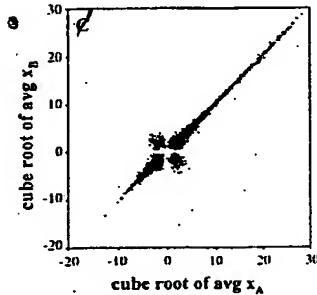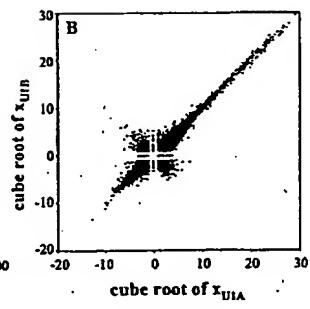
FIG. 1C   FIG. 1D

FIG.2A　　FIG.2B　　FIG.2C

FIG.2D　FIG.2E　FIG.2F

FIG. 3A

FIG. 3B

FIG. 3C

FIG. 4A

FIG. 4B

FIG. 4C

·cutoff   Gaussian weight function

FIG.5A   FIG.5B

FIG 6
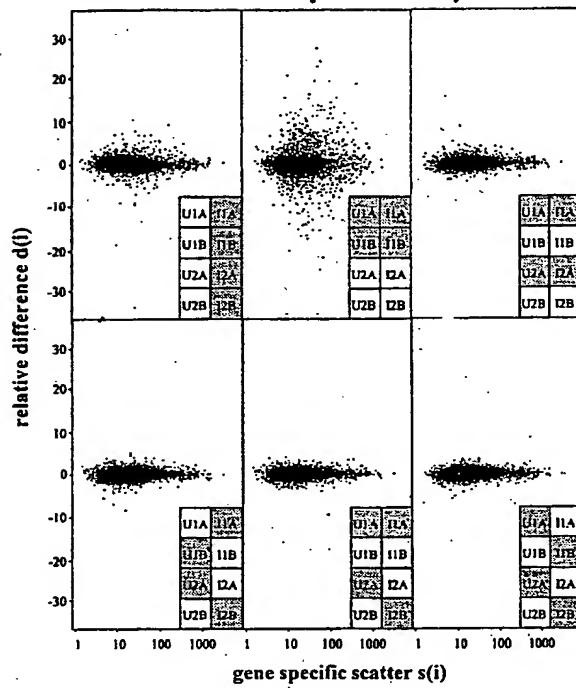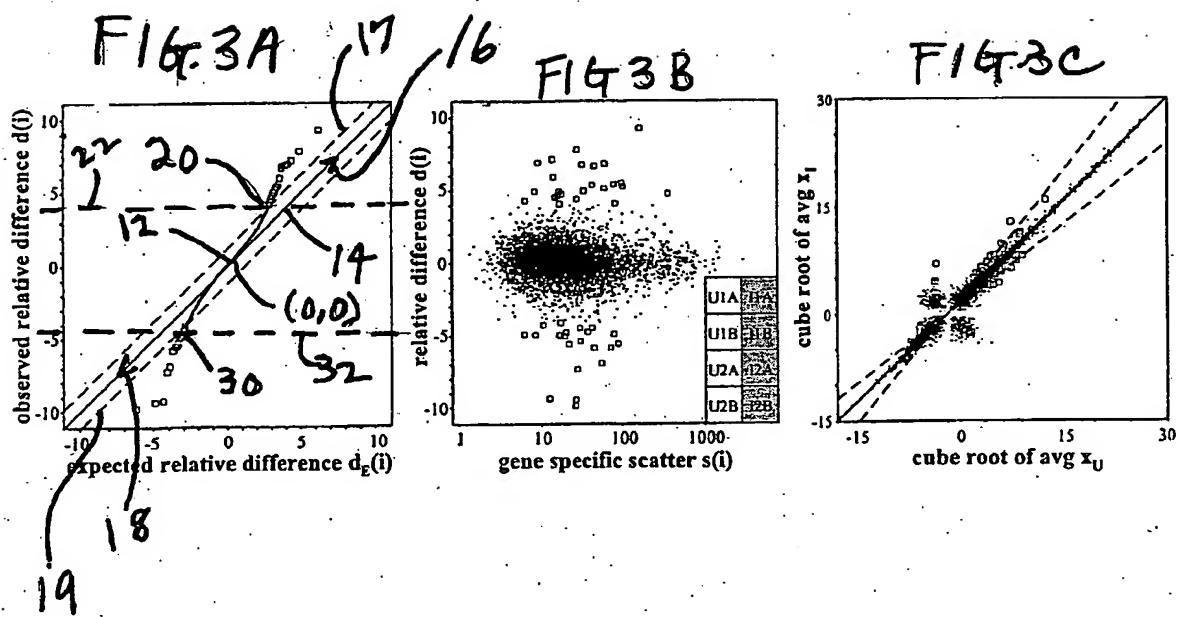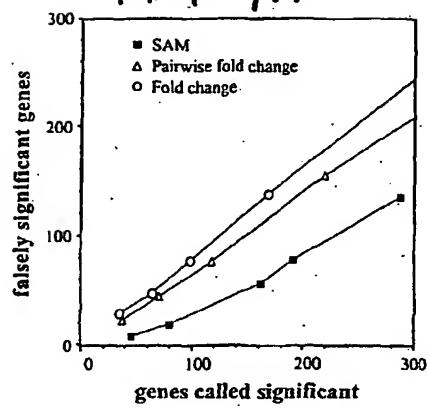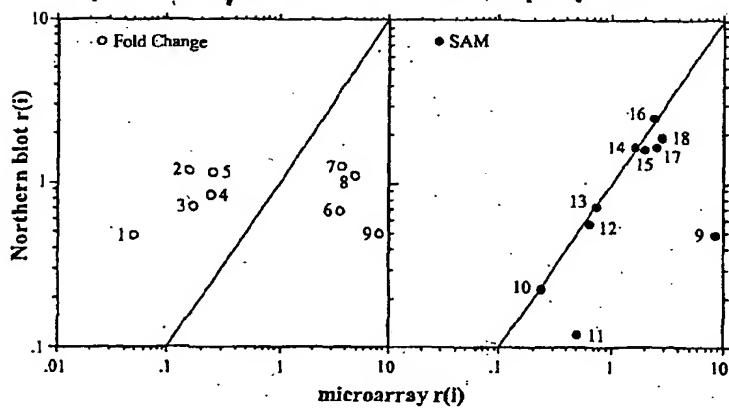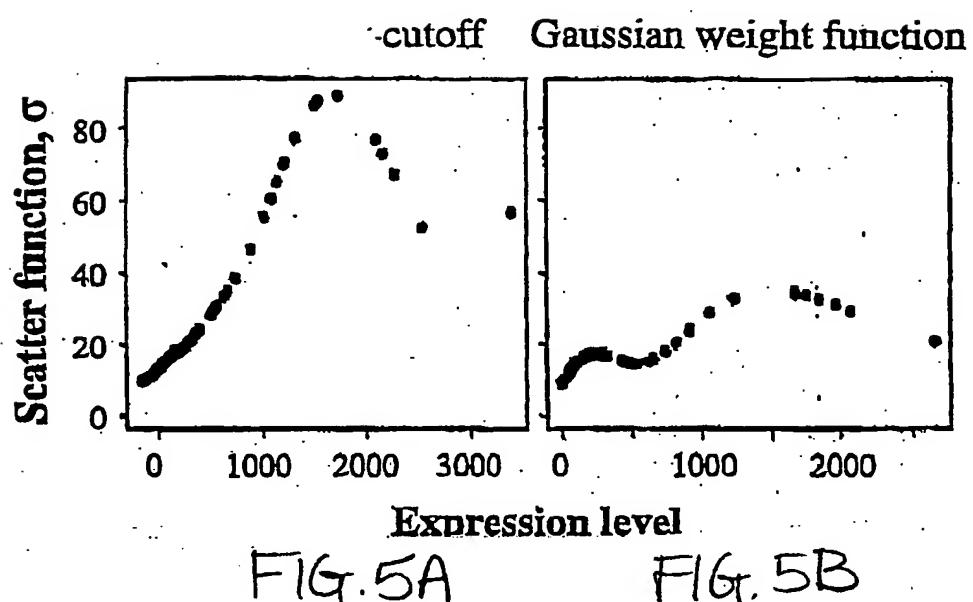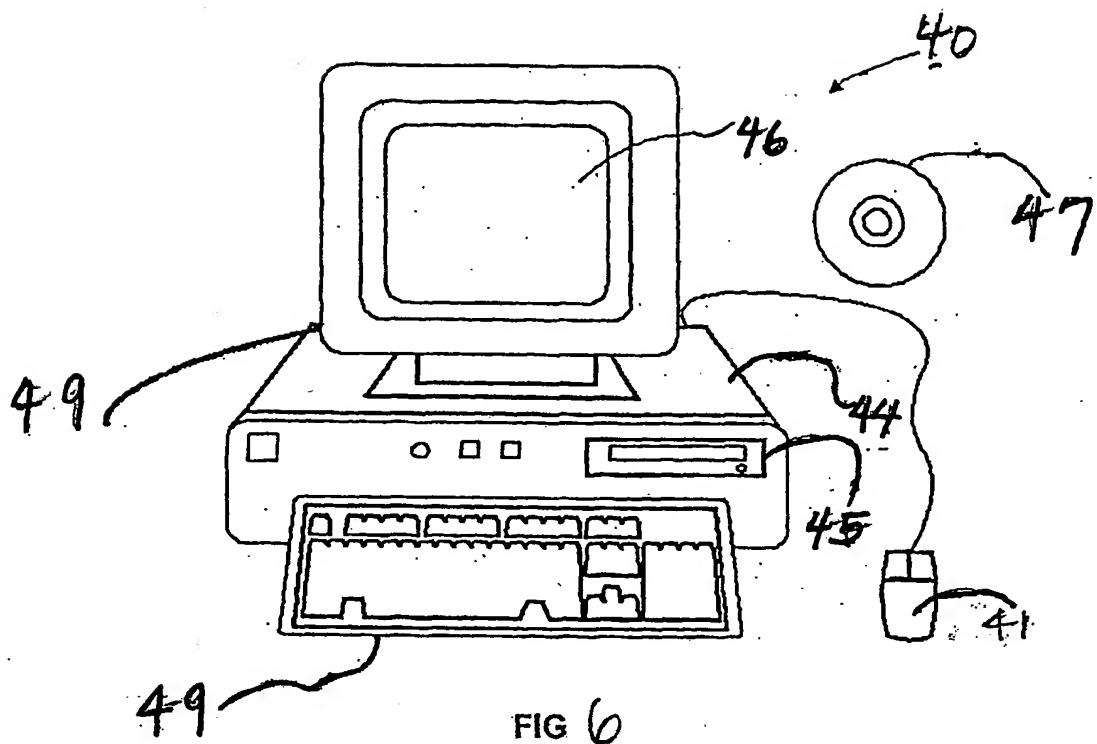
INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/14223

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : G01N 33/48; 33/50; C12Q 1/68; C12M 3/00
US CL : 702/19, 23; 435/6, 287.2, DIG51

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 702/19, 23; 435/6, 287.2, DIG51

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
USPatFull, Derwent, Medline, Scisearch

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 99/58720 A1 (ACACIA BIOSCIENCES INC.) 18 November 1999, see entire document and Figures 1, 5, and 6.	1-5, 11-13, 16, 17, 28-37, 44, 46-49, 51-56, 60-63 --------
X --- Y	EP 0935210 A2 (AFFYMETRIX INC.) 11 August 1999, see entire text and figure 5.	1-6, 28-33, 35, 36, 44 -------- 46-64
X	US 5,840,484 A (SEILHAMER et al.) 24 November 1998. See entire text.	1-6, 44-64
X, P	US 6,114,114 A (SEILHAMER et al.) 5 September, 2000. See entire text and Figures 1 and 3.	1-6
X, P	WO 00/28091 A1 (SCIOS INC.) 18 May 2000. See entire document and figures	1-64
X, P	WO 00/71756 A2 (AVENTIS PHARMACEUTICALS INC.) 30 November 2000. See entire text and figures.	1-64

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 July 2001 (27.07.2001)	3 1 AUG 2001

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Mary K Zeman TERRY J. DEY PARALEGAL SPECIALIST Telephone No. 703 308-0196 TECHNOLOGY CENTER 1600

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